

Serum adipocyte fatty acid binding protein as a new biomarker predicting the development of type 2 diabetes - a 10-year prospective study in Chinese

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Running title: A-FABP predicts development of type 2 diabetes

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Abstract

Objective Adipocyte fatty acid-binding protein (A-FABP) is abundantly expressed in adipocytes and plays a role in glucose homeostasis in experimental animals. We have previously shown that circulating A-FABP levels are associated with the metabolic syndrome, which confers an increased risk of type 2 diabetes (DM). Here we investigated whether serum A-FABP levels could predict the development of DM in a 10-year prospective study.

Research Design and Methods Baseline serum A-FABP levels were measured with ELISA in 544 non-diabetic subjects, recruited from the Hong Kong Cardiovascular Risk Factor Prevalence Study cohort, who were followed prospectively to assess the development of DM. The role of A-FABP in predicting the development of DM over 10 years was investigated using Cox regression analysis.

Results At baseline, serum sex-adjusted A-FABP levels were higher in subjects with impaired glucose tolerance or impaired fasting glucose (IGT/IFG) ($p < 0.00001$ versus normal glucose tolerance) and correlated positively with adverse cardio-metabolic risk factors. Over 10 years, 96 subjects had developed DM. High baseline A-FABP was predictive of DM, independent of obesity, insulin resistance or glycemic indices (RR 2.25, 95%CI 1.40-3.65; $p = 0.001$; above versus below sex-specific median). High A-FABP remained an independent predictor of DM in the high-risk IGT/IFG subgroup (adjusted RR 1.87, 95%CI 1.12-3.15, $p = 0.018$).

Conclusions Serum A-FABP was associated with glucose dysregulation and predicted the development of DM in Chinese.

Adipocyte fatty acid-binding protein (A-FABP), also known as aP2 or FABP4, is one of the most abundant proteins in mature adipocytes (1). It belongs to a family of fatty acid-binding proteins which are small cytoplasmic proteins expressed in a highly tissue-specific manner, thought to be important in mediating intracellular fatty acid trafficking and energy metabolism (2,3). Recent studies in animal models suggest that A-FABP may be important in glucose homeostasis. Deletion of A-FABP gene protected mice from insulin resistance and hyperinsulinemia associated with both diet-induced obesity (4) and genetic obesity (5). In humans, a promoter polymorphism, T-87C, of the A-FABP gene that resulted in reduced adipose tissue A-FABP mRNA expression was found to be associated with reduced risk for type 2 diabetes mellitus (DM) and cardiovascular disease (6).

We have previously demonstrated that A-FABP, although traditionally considered as an intracellular cytosolic protein, is present in the circulation (7). We have also reported the positive association between serum A-FABP levels and parameters of adiposity, hyperglycemia, insulin resistance and the metabolic syndrome (MetS) in cross-sectional (7,8) and longitudinal studies (9). As MetS is known to confer over 3-fold risk of developing DM (10), we set out to examine the role of serum A-FABP in predicting the risk of DM, using the data of subjects from the Hong Kong Cardiovascular Risk Factor Study who had just completed their 10-year follow-up.

Research Design and Methods

Subjects

Subjects were recruited from the population-based Hong Kong Cardiovascular Risk Factor Prevalence Study conducted in 1995-1996 (11), where unrelated individuals were randomly invited to undergo a comprehensive

assessment of cardiovascular risks, including a 75g oral glucose tolerance test (OGTT). In 1997, 322 subjects with impaired glucose tolerance (IGT) and 322 age- and sex-matched subjects with normal glucose tolerance (NGT) were invited to participate in a prospective study examining the progression to DM. All IGT subjects were given similar dietary and exercise advice without initiating active medical therapy at baseline, and all were under the care of their primary care physicians between assessments.

Subjects returned at years 2, 5 and 10 after an overnight 10-hour fast for a repeat OGTT assessment. For subjects with diagnosis of DM made and treatment started prior to follow-up assessment, date of DM diagnosis was ascertained and OGTT was not performed. Results from the year 2 and year 5 studies had been reported previously (9,12-14). For this report, subjects were classified as having NGT, IGT, impaired fasting glucose (IFG) or DM according to the WHO 1998 diagnostic criteria (15). Only subjects whose baseline fasting plasma glucose (FG) <7mmol/L and 2-hour post-OGTT glucose (2hG) <11.1mmol/L (non-diabetic according to the WHO98 criteria) and who had complete baseline anthropometric and biochemical data, were included in this report (5 subjects with baseline FG 7-7.8mmol/L were reclassified as DM and excluded from analysis. Subjects without baseline stored serum were also excluded). Altogether, 544 subjects were included in this report, of which 286 were NGT, 252 IGT and 6 IFG at baseline, after reclassification. No significant difference in baseline anthropometric parameters was found between the study cohort and subjects without available stored baseline serum. MetS was defined according to the US National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) guidelines (16), and modified as recommended in the latest American Heart Association/National Heart, Lung and Blood Institute Scientific Statement (17), by adopting the Asian criteria for abdominal

obesity (waist circumference [WC] ≥ 90 cm in men or ≥ 80 cm in women) and a lower cut-off for elevated fasting glucose (FG ≥ 5.6 mmol/L). MetS would be diagnosed in the presence of ≥ 3 of 5 adverse cardiometabolic factors: abdominal obesity, hypertriglyceridemia, reduced HDL, hypertension and elevated FG.

At each visit, medical histories were obtained. Family history of diabetes referred to first-degree relatives only. Alcohol drinking referred to any frequency of alcohol consumption including social drinking. Subjects were considered physically active when they had sessions of over half-hour of continuous exercise at least once per week. Anthropometric (body weight, height, BMI, WC, resting blood pressure [BP]) and biochemical parameters (FG, 2hG, insulin, total cholesterol, triglycerides [TG], LDL and HDL) were measured as described previously (9,11-14). Presence of hypertension was defined as BP $\geq 130/85$ mm Hg or on regular anti-hypertensive treatment. Insulin resistance was estimated using homeostasis model assessment index (HOMA-IR), calculated as (FG [in mmol/L] x fasting insulin [in mIU/L]/ 22.5). Serum hsCRP was measured using a particle-enhanced immunoturbidimetric assay (Roche Diagnostics, GmbH, Mannheim, Germany). Serum adiponectin was measured using an in-house sandwich ELISA established in our laboratory (14). A-FABP was measured using ELISA (BioVendor Laboratory Medicine, Inc., Czech Republic). Briefly, diluted serum samples, calibrators and quality-control samples were applied to 96-well microtitre plates coated with affinity-purified goat anti-human A-FABP antibody and the absorbance values were measured at 450nm. The intra- and inter-assay coefficients of variance were 3.7-6.4% and 2.6-5.3% respectively (7). All subjects gave informed consent, and the study was

approved by the Ethics Committee of the Faculty of Medicine, University of Hong Kong.

Statistical analyses

All statistical calculations were performed with SPSS (Chicago, IL) version 12.0. Results are presented as mean \pm SD or median with interquartile range as appropriate. Data with skewed distributions, as determined using Kolmogorov-Smirnov test, were logarithmically-transformed before analysis. Differences in baseline characteristics with glycemic status were compared using chi-square tests for categorical variables and one-way ANOVA for continuous variables. Bonferroni correction was used for multiple testing. Correlations between A-FABP and anthropometric and biochemical variables were analyzed using Pearson's correlation. Stepwise multiple logistic regression analysis was used to examine the association (odds ratio, OR) of baseline A-FABP with IGT/IFG at baseline. Population median of A-FABP was determined for each sex using the baseline data of the entire cohort of 544 subjects. The optimal sensitivity and specificity of using various cut-off values of A-FABP to predict DM was examined by the receiver operating characteristic curve (ROC) analysis. Survival was calculated from the date of visit at baseline to the date of diagnosis of DM or year-10 follow-up. Survivals were estimated by the Kaplan-Meier method and compared by the log-rank test. Subjects who were lost to follow-up were assumed to be free from DM at 10 years. To identify independent predictors of the development of DM, baseline variables that were significantly different between subjects with and without DM (after correction for multiple testing) and that were biologically likely to affect glycemic status, were analyzed using multiple Cox proportional hazard regression model with a stepwise elimination procedure. For parameters which were highly correlated, such as BMI and WC ($r=0.87$ in men and 0.79 in women; $p<0.001$), only one was entered into the regression analysis. Two-

sided *p* values of less than 0.05 were considered significant.

Results

There were 544 non-diabetic subjects with complete baseline demographic and biochemical data. Of these 286 were NGT and 258 were IGT/IFG at baseline. As expected, when compared to NGT subjects, those with IGT/IFG were more obese, more hyperinsulinemic and insulin resistant as estimated by HOMA-IR, more hypertensive, had more adverse lipid profiles and higher prevalence of MetS. They also had lower serum adiponectin, but higher hsCRP (all $p < 0.001$, IGT/IFG vs NGT). Consistent with our previous reports (7,9), A-FABP levels were significantly higher in women ($p < 0.001$). The sex-adjusted A-FABP concentrations were significantly higher in the IGT/IFG group (median of 18.2ng/ml vs 12.5ng/ml in men and 22.4ng/ml vs 18.5ng/ml in women; IGT/IFG vs NGT; $p < 0.00001$). There was no significant difference between the groups in smoking status, alcohol consumption, physical activity and the family history of DM.

Baseline sex-adjusted A-FABP levels correlated positively with increasing age, BMI, WC, systolic and diastolic blood pressure, FG, 2hG, fasting insulin, HOMA-IR, LDL-cholesterol, triglyceride and hsCRP, but negatively with HDL (all $p < 0.001$; data not shown). At baseline, the positive association of sex-adjusted A-FABP levels with IGT/IFG group remained significant (OR 2.05, 95%CI 1.38-3.04, $p < 0.001$) even after adjustment for age, BMI, HOMA-IR, adiponectin and hsCRP (data not shown).

Of the 544 subjects, 72 were lost to follow up at 5 years and 64 were lost to follow up at 10 years. The main reasons for their loss to follow-up were withdrawal of consent, emigration, change of address, disability or death. There were no significant differences in any baseline parameters

between subjects who returned for follow-up and those who did not, except for older age in those lost to follow-up. Over the 10 years, a total of 96 subjects (17.6%) had developed DM (21 new cases by 2 years, 26 by 5 years and 49 by 10 years). The cumulative 10-year DM incidence rate was 31.8% for those with baseline IGT/IFG and 4.9% for NGT.

The clinical characteristics of subjects who subsequently developed DM are shown in table 1. More men than women had developed DM in this cohort. As expected, those who had progressed to DM had more adverse cardio-metabolic risk factors at baseline, being more obese, insulin resistant, hyperglycemic, hypertensive and hyperlipidemic. They also had higher hsCRP, lower adiponectin levels, and higher prevalence of IGT/IFG and MetS at baseline (all $p < 0.001$, DM vs non-DM subjects). There was no significant difference between the two groups in the proportion of subjects on anti-hypertensive treatment at baseline (details of individual medications not available). There was also no significant difference in interval weight change between the two groups and no difference in crude physical activity assessment.

Baseline A-FABP levels were significantly higher in subjects who had developed DM by year 10 compared to those who did not (median of 19.3ng/ml vs 13.8ng/ml in men and 24.9ng/ml vs 19.2ng/ml in women, DM vs non-DM, $p < 0.00001$). On univariate analysis, the relative risk of A-FABP in predicting the development of DM was 3.03 (95% CI 2.00-4.60, $p < 0.00001$; table 1). All the well-known cardio-metabolic risk factors were all significantly associated with the development of DM in our cohort, together with high hsCRP and low adiponectin, on univariate analysis (table 1).

The Kaplan-Meier curve for the development of DM is shown in figure 1. Subjects with baseline A-FABP levels above the population median (according to sex) had significantly higher risk ($p < 0.00001$) of developing DM

compared to those with A-FABP levels below median. According to receiver-operator curve analysis, using the population median of 15.30ng/ml for men and 20.36ng/ml for women as cut-off offered sensitivities of 71% and 76% and specificities of 56% and 54% respectively which approximated to the optimal cut-off level of A-FABP in the prediction of DM.

The independent predictors of DM development were identified using stepwise multiple Cox proportional hazard regression model. As shown in table 2, baseline serum A-FABP was a significant independent predictor of DM development, together with male sex and baseline 2hG, in a model that included also age, baseline BMI, HOMA-IR, adiponectin and hsCRP. Subjects with A-FABP above the median had a relative risk of 2.25 (95%CI 1.40-3.65, $p=0.001$) of developing DM compared to those with A-FABP below the median at baseline. Identical results were obtained if WC was entered into the model instead of BMI, or if presence of MetS was added to the model. As the presence of IGT/IFG at baseline is known to be strongly associated with the development of DM, we also performed a subgroup analysis of this IGT/IFG cohort, and A-FABP remained a significant independent predictor of the development of DM ($p=0.018$), together with 2hG (RR 1.75, $p<0.001$) and adiponectin (RR 0.63, $p<0.05$), in a model including also age, sex, BMI, HOMA-IR and hsCRP. For IGT/IFG subjects with serum A-FABP above the median at baseline, the adjusted relative risk of developing DM over the course of 10 years was 1.87 (95%CI 1.12-3.15) compared to those with A-FABP below median.

Discussion

A-FABP, one of the most abundant proteins expressed in adipocytes, has recently been suggested to play an important role in energy metabolism. In this study, we have demonstrated that

circulating A-FABP levels were associated with dysglycemia and predicted the development of DM in a 10-year prospective study in Chinese. This is of potential clinical importance as the prevalence of DM is increasing worldwide, reaching epidemic dimensions in Asian countries (18).

The adverse effects of A-FABP on glucose metabolism in mice have been well demonstrated. In A-FABP-deficient mice, the improvement in glucose metabolism (4,5) was in part offset by the compensatory upregulation of mall, a minor isoform of FABP in adipocytes (4,19,20). On the other hand, mice with targeted disruption of both A-FABP and mall (aP2-mall^{-/-}) had better glucose tolerance and were more insulin sensitive than wild-type mice even on ordinary chow diet, and were protected from high fat diet-induced insulin resistance and DM (21). In addition, simultaneous ablation of A-FABP and mall in ob/ob mice led to normalization of hyperglycemia and associated metabolic abnormalities, even in the presence of obesity (22).

In humans, A-FABP expression was also found to affect glucose metabolism. A functional promoter polymorphism of the A-FABP gene, T-87C, which resulted in impaired transcriptional activation by CAAT box/enhancer-binding protein and hence reduced adipose A-FABP mRNA expression, was shown to be associated with a reduced risk for DM, especially in obese individuals (6). Our finding that high baseline serum A-FABP levels could predict the development of DM in our study subjects is in keeping with an adverse effect of increased A-FABP expression on glucose metabolism, as serum A-FABP level has been shown to correlate strongly with its protein expression in adipose tissues (9). The 10-year cumulative incidence rate of DM in our cohort was 17.6% (96/544), while those with A-FABP above and below the median at baseline had a cumulative incidence of 25.7% (70/272) and 9.6% (26/272) respectively. Assuming that the association between A-FABP and DM

was causal, then lowering A-FABP, if this were possible, would have substantial impact on the population incidence of DM. The dysregulated production of various adipokines, such as adiponectin and PAI-1, in obese individuals, has been shown to improve with weight reduction and various medications (23). The effect of weight reduction and other therapeutic approaches on A-FABP expression is currently being investigated in our laboratory.

The mechanism linking A-FABP with glucose homeostasis is not yet fully understood. It is known that A-FABP can bind various intracellular fatty acids and probably mediates intracellular lipid trafficking between cellular compartments (2). It can also form 1:1 complex with hormone sensitive lipase and enhance the efficiency of the lipase, hence facilitating lipolysis and efflux of fatty acids from adipocytes (24,25). It might also modulate the availability and composition of fatty acids in muscles and adipose tissues (26). In mice, improved glucose homeostasis in A-FABP ablation had been shown to be associated with preferential accumulation of shorter chain fatty acids in myocytes and adipose tissues. This was accompanied by increased AMP-activated protein kinase (AMPK) activity, Akt phosphorylation (26), and glucose oxidation in muscles (27), increased basal glucose conversion into lipids (28) and reduced lipolysis-induced insulin resistance and pancreatic dysfunction (20). While these observations might partly account for the enhanced insulin sensitivity observed in mice with A-FABP ablation, their physiological relevance in humans awaits further clarification.

We have recently demonstrated on proteomic analysis that A-FABP, traditionally considered a cytoplasmic protein, was released from cultured mature adipocytes in the absence of any obvious cell damage (7). In human, A-FABP circulates at concentrations of ~10-50

ng/ml, comparable or higher than that of most adipokines, and its level was elevated in obese subjects and correlated with BMI, WC, fat percentage and insulin resistance (7-9). In both Chinese (7-9) and Caucasians (29), close associations are found between serum A-FABP levels and various cardio-metabolic risk factors. We have also demonstrated that high serum A-FABP predicts the development of the MetS (9), well known to be associated with an increased risk of type 2 DM and cardiovascular diseases. In this 10-year prospective study, we have explored the association of circulating A-FABP levels with hyperglycemia, and found that baseline fasting A-FABP levels were elevated in subjects with IGT/IFG, compared to those with NGT. Furthermore, serum A-FABP level was predictive of the progression to DM, independent of the influence of established risk factors of DM, including excess adiposity (12), the presence of MetS (10) or IGT/IFG (10), and the more recently recognized predictors, such as hsCRP (13) and hypoadiponectinemia (30). Nevertheless, it is currently unclear whether circulating A-FABP functions as a lipid-hormone transporter or in a hormone-like fashion to mediate the effects on muscle glucose metabolism where no local A-FABP expression is present. Factors influencing the balance between serum and tissue A-FABP also remain to be determined. Further work investigating the possible physiological role of circulating A-FABP is in progress.

The current study population included only the IGT/IFG subjects who consented to participate in long-term follow-up, and their age- and sex-matched controls, instead of the entire population-based cohort of the Hong Kong Cardiovascular Risk Factor Prevalence Study (11). Our findings may therefore not be directly applicable to the general population due to the sampling bias introduced by the study design. Our study is also limited by the relatively small number of subjects with incident DM and the relatively high rate of loss to follow-up due to high rate of emigration, a common problem in prospective

studies in Hong Kong. Whether serum A-FABP can be useful in the prediction of DM needs to be confirmed in other populations and preferably with larger cohorts randomly recruited from the general population. As not all patients in Hong Kong see primary care physicians regularly, the documentation of the timing of incident DM in this cohort might be delayed and only diagnosed at the scheduled study follow-up (at 2, 5 or 10 years). More detailed data on diet, exercise and activity scores, which can impact on glycemic outcome, will also be helpful, although we did not observe a significant difference in the crude assessment of baseline physical activity level or interval weight changes between groups in the subjects studied. The lack of detailed data on concomitant medications is another limitation. The differential use of drugs which may affect glucose metabolism, such as angiotensin converting enzyme inhibitors or beta-blockers, cannot be excluded, although the proportion of subjects on anti-hypertensive medications was similar for those who did or did not

develop DM. Despite these limitations, our data suggest that the measurement of serum A-FABP level may be of potential clinical relevance in identifying subjects at risk of developing DM, independent of the well-established predictors including BMI, waist circumference, glucose or MetS.

In conclusion, we have demonstrated that circulating A-FABP levels were increased in subjects with glucose dysregulation and high A-FABP levels predicted the development of DM in this Chinese cohort. Further studies are warranted to examine the role of A-FABP in the pathogenesis of DM, and to investigate the potential application of this new biomarker for the identification of at-risk individuals for targeted lifestyle intervention, the benefit of which is well documented (31).

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References

1. Makowski L, Hotamisligil GS: Fatty acid binding proteins – the evolutionary crossroads of inflammatory and metabolic responses. *J Nutr* 134:2464S-2468S, 2004.
2. Coe NR, Bernlohr DA: Physiological properties and functions of intracellular fatty acid-binding proteins. *Biochem Biophys Acta* 1391:287-306, 1998.
3. Hertzel AV, Bernlohr DA: The mammalian fatty acid-binding protein multigene family: molecular and genetic insights into function. *Trends Endocrinol Metab* 11:175-180, 2000.
4. Hotamisligil GS, Johnson RS, Distel RJ, Ellis R, Papaioannou VE, Spiegelman BM: Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. *Science* 274:1377-1379, 1996.
5. Uysal KT, Scheja L, Wiesbrock SM, Bonner-Weir S, Hotamisligil GS: Improved glucose and lipid metabolism in genetically obese mice lacking aP2. *Endocrinology* 141:3388-3396, 2000.
6. Tuncman G, Erbay E, Hom X, De Vivo I, Campos H, Rimm EB, Hotamisligil GS: A genetic variant at the fatty acid-binding protein aP2 locus reduces the risk for hypertriglyceridemia, type 2 diabetes, and cardiovascular disease. *Proc Natl Acad Sci USA* 103:6970-6975, 2006.
7. Xu A, Wang Y, Xu JY, Stejskal D, Tam S, Zhang J, Wat NM, Wong WK, Lam KS: Adipocyte fatty acid binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. *Clin Chem* 52:405-413, 2006.
8. Yeung DCY, Xu A, Cheung CWS, Wat NMS, Yau MH, Fong CHY, Chau MT, Lam KSL. Serum adipocyte fatty acid-binding protein levels were independently associated with carotid atherosclerosis in Chinese women. *Arterioscler Thromb Vasc Biol* 2007. (in press)
9. Xu A, Tso AWK, Cheung BMY, Yu W, Wat NMS, Fong CHY, Janus ED, Lam KSL: Circulating adipocyte-fatty acid binding protein levels predict the development of the metabolic syndrome: a 5-year prospective study. *Circulation* 115:1537-1543, 2007.
10. Lorenzo C, Okoloise M, Williams K, Stern MP, Haffner SM: The metabolic syndrome as predictor of type 2 diabetes. The San Antonio Heart Study. *Diabetes Care* 26:3153-3159, 2003.
11. Janus ED, Wat NMS, Lam KS, Cockram C, Siu S, Liu L, Lam TH. On behalf of the Hong Kong Cardiovascular Risk Factor Steering Committee: The prevalence of diabetes, association with cardiovascular risk factors and implications of diagnostic criteria (ADA 1997 and WHO 1998) in a 1996 community-based population study in Hong Kong Chinese. *Diabetic Med* 17:741-745, 2000.
12. Wat NM, Lam TH, Janus ED, Lam KS. Central obesity predicts the worsening of glycemia in southern Chinese. *Int J Obes Relat Metab Disord* 25:1789-1793, 2001.
13. Tan KC, Wat NM, Tam SC, Janus ED, Lam TH, Lam KS: C-reactive protein predicts the deterioration of glycemia in Chinese subjects with impaired glucose tolerance. *Diabetes Care* 26: 2323-2328, 2003.
14. Tso AW, Sham PC, Wat NM, Xu A, Cheung BM, Rong R, Fong CH, Xu JY, Cheung KK, Janus ED, Lam KS: Polymorphisms of the gene encoding adiponectin and glycaemic outcome of Chinese subjects with impaired glucose tolerance: a 5-year follow-up study. *Diabetologia* 49:1806-1815, 2006.
15. Alberti KGMM, Zimmet P: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus: provisional report of a WHO consultation. *Diabetic Med* 15:539-553, 1998.
16. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA*. 285:2486-2497, 2001.
17. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Bordon DJ, Krauss RM, Savage PJ, Smith JrSC, Spertus JA, Costa F: Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 112:2735-2752, 2005.
18. Yoon KH, Lee JH, Kim JW, Cho JH, Choi YH, Ko SH, Zimmet P, Son HY: Epidemic obesity and type 2 diabetes in Asia. *Lancet* 368: 1681-1688, 2006.
19. Shaughnessy S, Smith ER, Kodukula S, Storch J, Fried SK: Adipocyte metabolism in adipocyte fatty acid binding protein knockout (aP2^{-/-} mice after short-term high-fat feeding - Functional compensation by the keratinocyte fatty acid binding protein. *Diabetes* 49:904-911, 2000.

20. Scheja L, Malowski L, Uysal KT, Wiesbrock SM, Shimshek DR, Meyers DS, Morgan M, Parker RA, Hotamisligil GS: Altered insulin secretion associated with reduced lipolytic efficiency in aP2^{-/-} mice. *Diabetes* 48:1987-1994, 1999.
21. Boord JB, Maeda K, Makowski L, Babaev VR, Fazio S, Linton MF, Hotamisligil GS: Combined adipocyte-macrophage fatty acid-binding protein deficiency improves metabolism, atherosclerosis, and survival in apolipoprotein E-deficient mice. *Circulation* 110:1492-1498, 2004.
22. Cao H, Maeda K, Gorgun CZ, Kim HJ, Park SY, Shulman GL, Kim JK, Hotamisligil GS: Regulation of metabolic responses by adipocyte/macrophage Fatty Acid-binding proteins in leptin-deficient mice. *Diabetes* 55:1915-1922, 2006.
23. Xavier PSF: The relationship of adipose tissue to cardiometabolic risk. *Clin Cornerstone* 8 (Suppl 4): S14-23, 2006.
24. Smith AJ, Sanders MA, Thompson BR, Londos C, Kraemer FB, Bernlohr DA: Physical association between the adipocyte fatty acid-binding protein and hormone-sensitive lipase: a fluorescence resonance energy transfer analysis. *J Biol Chem* 279:52399-52405, 2004.
25. Shen WJ, Sridhar K, Bernlohr DA, Kraemer FB: Interaction of rat hormone-sensitive lipase with adipocyte lipid-binding protein. *Proc Natl Acad Sci USA* 96:5528-5532, 1999.
26. Maeda K, Cao H, Kono K, Gorgun CZ, Furuhashi M, Uysal KT, Cao Q, Atsumi G, Malone H, Krishnan B, Minokoshi Y, Kahn BB, Parker RA, Hotamisligil GS: Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. *Cell Metab* 1:107-119, 2005 .
27. Baar RA, Dingfelder CS, Smith LA, Bernlohr DA, Wu C, Lange AJ, Parks EJ: Investigation of in vivo fatty acid metabolism in AFABP/aP2^{-/-} mice. *Am J Physiol Endocrinol Metab* 288:E187-193, 2005.
28. Coe NR, Simpson MA, Bernlohr DA: Targeted disruption of the adipocyte lipid-binding protein (aP2 protein) gene impairs fat cell lipolysis and increases cellular fatty acid levels. *J Lipid Res* 40:967-972, 1999.
29. Stejskal D, Karpisek M: Adipocyte fatty acid binding protein in a Caucasian population: a new marker of metabolic syndrome? *Eur J Clin Invest* 36:621-625, 2006.
30. Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA, Knowler WC, Krakoff J: Adiponectin and development of type 2 diabetes in the Pima Indian populations. *Lancet* 360: 57-8, 2002
31. Lindstrom J, Ilanne-Parikka P, Peltonen M, Aunola S, Eriksson JG, Hemio K, Hamalainen H, Harkonen P, Keinanen-Kiukaanniemi S, Laakso M, Louheranta A, Mannelin M, Paturi M, Sundvall J, Valle TT, Uusitupa M, Tuomilehto J; Finnish Diabetes Prevention Study Group: Sustained reduction in the incidence of type 2 diabetes by lifestyle intervention: follow-up of the Finnish Diabetes Prevention Study. *Lancet* 368: 1673-1679, 2006

Table 1. Baseline clinical parameters of subjects, with or without developing DM by 10 years, and the relative risk of each parameter in the prediction of the development of DM over a median of 10.0 years using univariate Cox regression analysis.

	DM	Non-DM	P value	Relative risk	95% CI	P value
N	96	448	--	--	--	--
Age (years)	51.2 ± 11.8	50.7 ± 12.5	0.745	1.02	1.00 – 1.03	0.037
Men/Women	51 / 45	183 / 265	0.027	1.33	1.10 – 1.63	0.005
BMI (kg/m ²)	26.4 ± 3.0	24.3 ± 3.7	<0.001	1.14	1.08 – 1.19	<0.001
Waist circumference (cm)	M: 89.5 ± 7.8 F: 82.2 ± 8.8	83.4 ± 9.5 76.6 ± 8.8	<0.001‡	1.06	1.04 – 1.08	<0.001‡
Systolic blood pressure (mmHg)*	128 ± 18.8	119 ± 17.7	<0.001	1.03	1.02 – 1.04	<0.001
Diastolic blood pressure (mmHg)*	80 ± 10.2	74 ± 9.6	<0.001	1.05	1.03 – 1.08	<0.001
Mean arterial pressure (mmHg)*	96 ± 12.4	89 ± 11.5	<0.001	1.05	1.03 – 1.07	<0.001
Hypertension (%)	54.2	33.7	<0.001	2.49	1.66 – 3.73	<0.001
Fasting glucose (mmol/L)	5.5 ± 0.5	5.1 ± 0.5	<0.001	3.38	2.31 – 4.95	<0.001
2-hr post-OGTT glucose (mmol/L)	8.8 ± 1.5	6.8 ± 1.8	<0.001	2.00	1.72 – 2.33	<0.001
Insulin (mIU/L)†	6.9 (4.6 – 10.5)	5.0 (3.3 – 7.3)	<0.001	1.96	1.40 – 2.73	<0.001
HOMA-IR for insulin resistance†	1.7 (1.1 – 2.7)	1.1 (0.7 – 1.7)	<0.001	2.09	1.51 – 2.85	<0.001
LDL-cholesterol (mmol/L)	3.6 ± 1.0	3.3 ± 0.9	0.004	1.39	1.12 – 1.72	0.003
HDL-cholesterol (mmol/L)	1.1 ± 0.3	1.3 ± 0.3	<0.001	0.22	0.11 – 0.44	<0.001
Triglyceride (mmol/L)†	1.4 (1.0 – 1.9)	1.0 (0.7 – 1.5)	<0.001	2.51	1.83 – 3.44	<0.001
Adiponectin (µg/ml)†	M: 4.2 (2.5 – 6.3) F: 5.0 (3.7 – 7.3)	5.5 (3.7 – 7.4) 6.6 (4.9 – 9.3)	<0.001‡	0.50	0.35 – 0.71	<0.001‡
High sensitivity CRP (mg/L)†	1.4 (0.7 – 2.6)	0.8 (0.3 – 1.8)	0.001	1.56	1.29 – 1.87	<0.001
Adipocyte fatty acid-binding protein (ng/ml)†	M: 19.3 (13.5 – 24.4) F: 24.9 (19.0 – 28.9)	13.8 (9.3 – 19.9) 19.2 (12.7 – 27.0)	<0.00001‡	3.03	2.00 – 4.60	<0.00001‡

Presence of IGT/IFG (%)	85.4	39.3	<0.001	8.37	4.74 – 14.8	<0.001
Presence of metabolic syndrome (%)	43.8	19.2	<0.001	2.95	1.97 – 4.42	<0.001

Data are mean±SD or median with interquartile range. * Excluded 56 subjects on anti-hypertensives. †Log-transformed before analyses. ‡Sex-adjusted p-value.

Table 2. Baseline predictors of the development of DM over a median 10.0 years of follow-up, examined using multiple stepwise Cox regression analysis (final model).

Baseline parameters	Relative risk	95% CI	P value
Sex (Male)	1.30	1.05 – 1.61	0.017
2-hr post-OGTT glucose	1.84	1.57 – 2.15	<0.001
Adiponectin*	0.69	0.45 – 1.05	0.081
Sex-specific A-FABP†	2.25	1.40 – 3.65	0.001

*Log-transformed before analysis. †Above vs. below sex-specific median, with the latter as reference with RR=1. Model included sex, age, baseline BMI, 2hr post-OGTT glucose, HOMA-IR, adiponectin, hsCRP and A-FABP in sex-specific median.

Figure 1 Cumulative survival (by the Kaplan Meier method) for the development of DM over a median follow up of 10.0 years for subjects with A-FABP above and below sex-specific median.

